Passage of Nucleopolyhedrosis Virus by Avian and Mammalian Predators of the Gypsy Moth, *Lymantria dispar*[1,2,3]

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ABSTRACT

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Five species of mammals and 3 species of birds passed polyhedral inclusion bodies (PIB) of the gypsy moth nucleopolyhedrosis virus (NPV) through their alimentary tracts in amounts great enough to kill gypsy moth larvae in bioassays. Opossums and raccoons passed roughly 5% of the PIB administered by intubation while white-footed mice, a short-tailed shrew, and southern flying squirrels passed 2.3, 1.8, and 0.05%, respectively. House finches and a red-winged blackbird passed close to 15% of the PIB administered, whereas mourning doves passed 0.05%.

All the birds, as well as the shrew and squirrels, passed the PIB within 6 h of intubation. The white-footed mice passed PIB within 18 h of intubation, while it took the raccoons 22, and the opossums 70 h to eliminate PIB from their alimentary tracts.

It is concluded that both mammals and birds pass significant amounts of NPV, and that both groups have features which contribute to their ability to passively transport NPV within the environment.

Lautenschlager and Podgwaite (1977) have shown that 2 wild mammal predators of the gypsy moth, the white-footed mouse, *Peromyscus leucopus* Rafinesque, and the short-tailed shrew, *Blarina brevicauda* Say, can pass significant amounts of nucleopolyhedrosis virus (NPV) of the gypsy moth, *Lymantria dispar* L., through their alimentary tracts. They suggested that these mammals might be important in circulating NPV within their home ranges, and that dispersing animals could introduce NPV into areas previously free of NPV.

Several researchers have indicated that NPV can successfully pass through the alimentary tracts of a variety of birds (Franz et al. 1955, Bird 1955, Hostetter and Biever 1970). Recently, Entwistle et al. (1977a, b) demonstrated that 16 species of birds disperse infectious NPV of the European spruce sawfly, *Gilpinia hercyniae* Hartig. These researchers also found that during the insect's nonlarval period, certain of these bird species continued to pass infectious NPV that they had apparently ingested while feeding on the corpses of NPV-killed larvae adhering to trees. Further, they found that these birds dispersed active NPV up to 6 km away from NPV-contaminated areas.

Since both wild birds and mammals are potentially important in the translocation of gypsy moth NPV within their immediate habitats, as well as into previously uninfected areas, we attempted to determine (1) species that can pass significant amounts of active NPV; (2) the proportion of ingested NPV that was passed and remained active; (3) passage rate of NPV (i.e., length of time after ingestion of NPV until all NPV was eliminated from the alimentary tract); (4) relation of passage rate to loss of NPV activity; and (5) the amount of NPV passed by mice after feeding on NPV-infected gypsy moth larvae.

Methods and Materials

Species and Numbers

All birds and mammals in these experiments were wild-trapped or netted in Connecticut and then transferred to cages in a walk-in environmental chamber that was set to simulate late spring conditions (8 h dark at 18°±1°C; 16 h light at 26°±1°C). Birds and mammals represented several distinct size groups. All mammals had been established as gypsy moth predators (Smith and Lautenschlager 1978). Bird species were chosen for ease of capture and adaptability to caged conditions. Although the bird species chosen are not recognized as important gypsy moth predators, they represent the size of birds that are important predators.

The numbers of test and control animals of each species were: the white-footed mouse, 3 and 2; the short-tailed shrew, 1 and 1; the southern flying squirrel, *Glaucomys volans* L., 2 and 2; the Virginia opossum, *Didelphis marsupialis* L., 3 and 2; the raccoon, *Procyon lotor* L., 3 and 2; the house finch, *Carpodacus mexicanus* Muller, 3 and 2; the red-winged blackbird, *Agelaius phoeniceus* L., 1 and 1; and the mourning dove, *Zenaidura macroura* L., 3 and 2.

Mammals were maintained on diets of water and either laboratory mouse chow or canned dog food; birds were maintained on diets of water and wild bird seed.

NPV Intubation Techniques

Each test white-footed mouse, short-tailed shrew, and flying squirrel received 0.1 ml of an aqueous suspension containing 1.25×10^9 purified polyhedral inclusion bodies (PIB) (Breillatt et al. 1972) of the gypsy moth NPV. The dose, which approximated the number of PIB produced in one NPV-infected 5th-stage gypsy moth larva, was administered directly into the stomach through a curved ball-end intubation needle. Control animals were intubated with 0.1 ml of sterile distilled water. All small mammals were individually housed in 30×17×10-cm plastic cages containing a nest chamber and an activity chamber. Following intubation, feces were collected

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from these cages at 2-h intervals for 48 h, and stored in sterile containers at 4°C.

Each test opossum and raccoon was anesthetized by an intramuscular injection of an appropriate dose of Ketalar® (ketamine hydrochloride)[5] and then treated with 2.5×10^{10} PIB in a powdered lactose carrier. The dose was in a modified gelatin capsule that was placed directly in the stomach with an intubation tube. Control opossums and raccoons were anesthetized and intubated with a capsule containing only powdered lactose. Test and control animals were individually housed in $1.5\times0.3\times0.75$-m wire mesh cages with attached wooden nest boxes. Feces from large mammals were collected at 6, 13, 22, 27, 30, 46, 54, 70, and 94 h after intubation and stored at 4°C in sterile 0.47-liter containers.

Test and control birds were not anesthetized but were intubated with modified gelatin capsule similar to that used for the large mammals. Each test bird received 1.25×10^{9} PIB in powdered lactose. Control birds received only powdered lactose. Birds were individually caged in 0.9-m^3 wire mesh cages with a stack of sterile plastic sheets tacked to the floor. Every 2 h for a series of 48 h, the top sheet was removed and cleaned of feces, which were stored at 4°C.

Examination of Feces for PIB

The amount of PIB passed in the feces of small mammals and birds was determined by counting PIB in fecal suspensions by use of a hemacytometer and 400X light optics. Blind counts of all PIB were made on feces collected from NPV treated and control animals. To prepare a feces sample for PIB counts, feces were dried for 24 h at 24°±1°C in a desiccator over Silica-gel. Dried samples, which ranged in weight from 3.7–117.1 mg for small mammals, and from 10.7–203.2 mg for birds, were mixed in a tissue homogenizer with sterile distilled water (1:70 w/v). Each thoroughly homogenized fecal suspension was serially diluted and the appropriate dilution was mixed and counted with a Levy-Neubauer® hemacytometer. This procedure was replicated 4 times for each sample. Mean PIB counts and standard errors were calculated for each test and control suspension. To eliminate counts of PIB-like debris and spores from the final calculations, the mean PIB-like particulate counts of control suspensions of a species were subtracted from the mean count of each test suspension of that species, and the number of PIB per mg feces was determined.

One-half of the homogenized fecal suspensions from test and control small mammals and birds were bioassayed for activity against 5 groups of 10 newly molted 2nd-stage gypsy moth larvae. For each group, 0.05 ml of a feces suspension was applied to the surface of an artificial diet block (pH 6.4–7.0); the larvae were maintained for 14 days by the procedure outlined by Lautenschlager and Podgwaite (1977). If mortality exceeded 90%, fecal suspensions were diluted and bioassayed until mortality was below 90%. The results of the bioassays, plotted on a regression line generated from mortality caused by bioassays of known amounts of PIB mixed in control mammal feces, yielded estimates of the PIB content in each sample.

The wet wt of feces samples from the large mammals ranged from 8.5–113.5 g and contained too much bulk to be counted with the hemacytometer. These samples were prepared for bioassay by mixing the entire feces sample with sterile distilled water (1:8 w/v), then homogenizing it in a sterile Waring® blender. Thoroughly homogenized samples were bioassayed against gypsy moth larvae as described for small mammals and birds.

The following additional tests were conducted to determine if small mammals passed intact infectious PIB after feeding on NPV-infected gypsy moth larvae, and if either healthy or diseased gypsy moth larvae were preferred by small mammals. Fifty 5th-stage NPV-infected larvae were offered to each of 10 white-footed mice. At the same time, 10 control mice were each offered 50 healthy 5th-stage larvae. Each NPV-infected larva was in its 10th day of infection and contained a minimum of 1×10^{9} PIB. Larvae were placed in the activity chamber of each mouse's cage along with artificial diet. After 20 h, the number of larvae remaining in each cage was determined and feces were collected from the nest chamber and stored at 4°C. Feces were not collected from the activity chamber to avoid probable contamination from NPV-infected larvae in that chamber. Feces from 5 test and 5 control mice that had eaten the most larvae (10–47) were examined for PIB and bioassayed for NPV activity by the methods described above.

Results

All animals tested, including those fed NPV-infected gypsy moth larvae, passed intact infectious PIB through their alimentary tracts. However, the amount passed varied significantly within and among species. In all cases, bioassay data on fecal suspensions revealed a close correlation (within one standard error) between the number of PIB observed in hemacytometer counts and the number of PIB required to cause a particular mortality percentage in standard bioassays. Therefore, we concluded that PIB counts were accurate and that the PIB counted were infectious.

The count of PIB passed and the rate of passage for small mammals (Table 1) showed that white-footed mice and the short-tailed shrew passed greater amounts than the southern flying squirrel. This was probably related to the rapid metabolic rate of these 2 small mammals. The PIB were probably moved rapidly through the acidic stomachs of these mammals into a more favorable pH environment in the intestines. The flying squirrels began to pass the PIB 2 h later than the mice and shrew, indicating that the PIB remained in their alimentary tracts longer than in the other smaller mammals.

Test white-footed mice ate 17.45±4.05[6] of the NPV-infected larvae while control mice ate 10.90±3.34 of the healthy larvae. Feces collected from the mice that had eaten the infected larvae contained intact, infectious PIB. The amount of PIB passed by these mice was roughly proportional to the number of larvae an individual mouse had eaten. Although the percentage of the amount of PIB consumed and then passed by each mouse was not precisely determined, it approximated

[5] The use of trade, firm, or corporation names in this paper is for the information and convenience of the reader. Such use does not constitute an endorsement or approval by the USDA or the Forest Service of any product or service to the exclusion of others that may be suitable.

[6] Mean and standard error.

Table 1.—Polyhedral inclusion bodies (PIB)[a] passed through the alimentary tracts of small mammals.[b]

Hours post intubation	White-footed mouse			Short-tailed shrew	Southern flying squirrel	
	40.0 g	13.0 g	23.2 g	23.4 g	72.1 g	56.1 g
2	0.0	0.3±0.10	0.2±0.10	22.7±0.01	0.0	0.0
4	—	12.0±2.50	0.2±0.14	0.7±0.07	0.6±0.44	0.7±0.07
6	37.5±1.30	7.86±0.67	0.8±0.16	trace	0.0	0.0
8	15.5±0.80	1.24±0.24	1.1±0.43	—	0.0	0.0
10	3.0±0.21	0.3±0.12	3.3±0.51	0.0	0.0	0.0
12	0.6±0.02	0.0	0.4±0.09	0.0	0.0	0.0
14	0.3±0.05	0.0	0.0	0.0	0.0	0.0
16	—	0.0	0.0	0.0	0.0	0.0
18	0.2±0.06	0.0	0.0	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0	0.0	0.0
Total	57.1±2.44	21.7±3.63	6.0±1.43	23.4±0.08	0.6±0.44	0.7±0.07
%	4.57	1.74	0.42	1.87	0.05	0.05

[a] Mean and SE hemacytometer counts ($\times 10^6$) verified by bioassay.
[b] Each animal intubated with 1.25×10^9 PIB.

Table 2.—Polyhedral inclusion bodies (PIB)[a] passed through the alimentary tracts of large mammals.[b]

Hours post intubation	Opossum			Raccoon		
	2.61 kg	2.27 kg	2.50 kg	2.61 kg	4.43 kg	2.50 kg
6	—	—	—	—	0.31±0.07	0.0
13	4.04±2.63	—	—	0.58±0.18	0.10±0.09	0.98±0.31
22	—	0.0	0.0	0.86±0.19	—	37.10±6.20
27	—	—	—	—	—	—
30	—	—	—	—	0.0	0.0
46	22.60±0.30	0.21±0.18	4.92±3.69	0.0	—	0.0
54	—	—	—	0.0	0.0	0.0
70	7.49±6.13	0.21±0.08	0.42±0.25	0.0	0.0	0.0
94	0.0	0.0	0.0	0.0	0.0	0.0
Total	34.13±9.06	0.42±0.26	5.43±3.94	1.44±0.37	0.41±0.16	38.08±6.51
%	13.64	0.17	2.14	0.58	0.16	15.24

[a] Number of PIB ($\times 10^8$) (Mean ± SE) based on standard bioassays.
[b] Each animal intubated with 2.5×10^{10} PIB.

Table 3.—Polyhedral inclusion bodies (PIB)[a] passed through the alimentary tracts of birds.[b]

Hours post intubation	House finch			Redwing blackbird	Mourning dove		
	23.5 g	17.9 g	19.7 g	51.0 g	104.1 g	117.0 g	104.8 g
2	6.12±0.38	42.20±5.10	2.75±0.34	0.16±0.06	0.0	0.0	0.0
4	0.0	0.0	0.0	18.80±1.40	0.12±0.09	0.53±0.08	0.99±0.06
6	0.0	0.0	0.0	0.0	0.39±0.10	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	6.12±0.38	42.20±5.10	2.75±0.34	18.96±1.46	0.51±0.19	0.53±0.08	0.99±0.06
%	4.90	33.76	2.21	15.20	0.04	0.04	0.08

[a] Mean and SE hemacytometer counts ($\times 10^7$) verified by bioassay.
[b] Each bird intubated with 1.25×10^9 PIB.

the passage time and percentage outlined for mice intubated with known amounts of purified PIB. Therefore, we assume that in the wild, species that ingest infected gypsy moths would show passage rates and amounts similar to those of the caged animals intubated with known amounts of PIB.

The larger mammals (Table 2) passed a mean of 5.3% of the amount of PIB that they ingested and passed it more slowly than the small mammals. Raccoons passed the PIB within 22 h but the opossums took over a 70-h period, although the amount passed by both species was virtually the same.

The pH of stomach contents of raccoons and opossums was closer to neutral (4.0–6.0) than was the pH of the stomachs of small mammals (2.0–5.0). Since PIB normally degrade at pH levels above 8.0 and below 3.1 (Bergold 1953), this higher pH along with the larger amount of bulk ingested by the large mammals probably presented an environment more favorable to the survival of PIB.

Passage of PIB through birds is shown in Table 3. The time from intubation until PIB were first seen in the feces was longest for the mourning doves. This species also passed the smallest amount—0.05%. The smallest birds (house finches) passed larger amounts, a mean of 13.4% of the PIB, with one house finch passing 34%. Only one red-winged blackbird was available for this experiment and this bird passed 15.2% of the PIB fed. However, this bird was diarrheic and we assume it normally would have exhibited a slower passage rate and would have passed fewer active PIB.

Discussion

All the animals tested passed PIB in amounts large enough to cause significant mortality if ingested by gypsy moth larvae. The amount of PIB passed by the birds, like the amount passed by the small mammals, seemed to be related to size, with the larger species passing the least and the smaller species passing the most.

The results indicate that certain species may be more important in the movement of NPV than others. For several reasons, the white-footed mouse may be important in transporting gypsy moth NPV. This mammal is an important gypsy moth predator (Smith and Lautenschlager 1978), exhibits a considerable degree of arboreal adaptation (Horner 1954, M'Closkey 1975), and prefers NPV-infected gypsy moth larvae over healthy larvae. However, the short-tailed shrew, which would contact larvae or pupae in the litter, might also disperse NPV within the shrews' home range, or move it farther during dispersal.

The small amount passed by the flying squirrels coupled with the fact that squirrels probably eat few gypsy moth larvae (Smith and Lautenschlager 1978) suggests that squirrels have low potential for dispersing NPV.

The large mammals passed significant amounts of NPV over a relatively long period. The extensive period measured for the opossum is probably related to the lower metabolic rate and long alimentary tract of this "primitive" mammal. This extended period could make the opossum an important source of NPV movement.

Birds clearly demonstrate a great potential for transporting NPV. Birds have great mobility and pass large amounts of PIB, and their high metabolic rate creates a constant need for food. Also, birds prefer insects infected with NPV (Hostetter and Biever 1970), and they are much more likely than mammals to deposit NPV on foliage where gypsy moth larvae may ingest it. Entwistle et al. (1977b) felt that "birds may be the single most important carriers of [NPV] inoculum over long distances," and that they may be "substantial contributors to more local movement." Also, Entwistle et al. (1978), in a study of the effects of NPV on the European spruce sawfly, *Gilpinia hercyniae* Hartig, recently showed that although the peak passage of NPV occurred in less than 1 h after the birds ate infected larvae, bioassays of the fecal material indicated that 6 of 10 birds continued to pass infectious NPV on the 3rd day after ingestion; one bird continued to pass infectious NPV on the 7th day.

Mammals are also important carriers of NPV inoculum. Mammals passed active NPV more slowly through their alimentary tracts than birds. This slower passage may enable mammals to transport NPV a considerable distance. Also, in any given area of northeastern forests, mammal populations are normally significantly larger than bird populations (Smith and Lautenschlager 1978). Therefore, mammals may collectively pass more NPV than birds.

This study was designed to quantify the amount and time over which PIB were passed in a laboratory situation. Examination of field data from another study[7] indicates that free-living mammals ingest gypsy moth NPV that remains active in their alimentary tracts. In fact, roughly 75% of the alimentary tracts taken from 169 mammals collected from NPV-epizootic areas contained enough NPV to cause gypsy moth mortality.

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